least an amino acid immediately preceding the alpha helix and at alpha helix positions +1, +2 and +6 of at least one zinc finger;

b) expressing the library in a suitable host cell; and

c) isolating a zinc finger protein that binds to a desired target nucleic acid, or a nucleic acid encoding the zinc finger protein.

48. (New) The method of claim 47, wherein the expression library is a phage display library.

49. (New) The method of claim 47, wherein the variant zinc finger proteins differ from each other in at least an amino acid immediately preceding the alpha helix and at alpha helix positions +1, +2 and +6 of at least two zinc fingers.

50. (New) The method of claim 47, wherein the variant zinc finger proteins differ from each other by random substitutions.--

II. REMARKS

Upon entry of the amendment, claims 2 to 5, 16 to 19, 40 and 42-50 will be pending. Claims 3 and 40 have been amended and claims 6 to 15, 20 to 39 and 41 have been cancelled without prejudice. For the Examiner's convenience, a marked-up copy of the claims is attached hereto as "Version with Markings to Show Changes Made." New claims 42-50 find support in the originally filed patent application and claims. No new matter has been added.

A. Regarding the Amendments

Claim 3 has been amended to remove the term "derived from" in that claim. This amendment was made to clarify the claim and is supported in the specification, for example, at page 10, lines 16-24.

Claim 40 was amended to independent form. Previous claim 40 was dependent on claim 27, a non-elected claim. Claim 40, as amended, contains the original subject matter of claim 27. Therefore, this amendment is supported by original claims 27 and 40.

Amendment of the claims is not to be construed as acquiescence to any of the rejections/objections set forth in the instant Office Action or previous applications, and was done solely to expedite prosecution of the instant application. Applicant hereby reserves the right to prosecute the claims as originally filed, or similar claims, in one or more continuation applications.

B. Regarding the Sequence Listing

In the Supplemental Office Action mailed December 05, 2000, the Examiner stated that the application failed to comply with the requirements of 37 CFR 1.821 through 1.825 because sequences were set forth that lack sequence identifiers. Given as examples were the sequences on page 51, lines 16-24 and in Figures 1 and 2. These sequences are fully disclosed in the specification. Amendments have been made to the specification, as listed above, to clearly indicate where in the Sequence Listing each of the sequences is listed. A Sequence Listing was originally submitter to the on February 9, 2000, with the filing of the present application. However, filed with this response is a new Sequence Listing. All sequences listed in the specification now contain references to the SEQ ID Nos in this newly filed Sequence Listing. As such, it is respectfully submitted that the present application is in compliance with the requirements of 37 C.F.R § 1.821 to 1.825.

C. Regarding the Election in Response to the Restriction Requirement

In response to the Restriction Requirement set forth in Paper No. 6, Applicant elects, with traverse, Group I, consisting of Claims 2 to 5, 16 to 19, and 40 drawn to zinc finger-nucleotide binding polypeptide variants. This election is an affirmation of the telephonic provisional election made on June 1, 2000, with traverse, to prosecute the invention of Group I.

D. Rejection for Double Patenting

Applicant acknowledges the alleged double patenting rejections of claims 2 to 5, 16 to 19 and 40 over claims 1 to 7, 21 to 22 and 53 of issued U.S. Patent No. 6,140,466 and over claim 1 of U.S. Application No. 09/500,691. However, Applicants respectfully defer responding to these rejections until the claims of the subject application otherwise are in a condition for allowance.

E. Rejection under 35 U.S.C. § 112

The objection to the specification and the corresponding rejections of claims 16 and 17 under 35 U.S.C. § 112, first paragraph, as allegedly lacking an adequate written description are respectfully traversed.

The specification is objected to as allegedly not providing support for the terms "at least three zinc finger modules" and "at least five finger modules." It is submitted, however, that the specification clearly supports these phrases. In the specification, for example, at page 11, lines 28-30, it is stated that "[t]he zinc finger-nucleotide binding polypeptide variant of the invention comprises at least two and preferably at least about four zinc finger modules that bind to a cellular nucleotide sequence and modulate the function of the cellular nucleotide sequence." Additionally, Example 1 discusses use of a recombinant polypeptide with at least three TFIIIA zinc fingers which contain histidine and arginine at the DNA contact positions. Similarly, Example 2 uses a zif268 three finger protein. These examples show that at the time of the filing of the invention the inventor had possession of the claimed invention with respect to polypeptides with at least three fingers. Accordingly, it is respectfully requested that the objection to the specification be withdrawn and that the corresponding rejections of claims 16 and 17 under 35 U.S.C. §112, first paragraph, as lacking an adequate written description be removed.

With respect to claim 3, it is alleged that the term "derived from" is unclear. As set forth in the amendments above, this term has been removed from claim 3. As amended, claim 3 is fully supported by the specification. Claim 3 claims a variant, as defined in the specification at

page 10, lines 16-24. As set forth in the specification, a variant is "a polypeptide which is a mutagenized form of a zinc finger protein or one produced through recombination." In the present application a variant is used, as opposed to use of a wild-type polypeptide. As such, it is respectfully submitted that the rejection of claim 3 is now moot.

With respect to claim 40, it is alleged that the claim is unclear because it is dependent on a claim that is no longer pending. As suggested by the Examiner, claim 40 has been amended to independent form, by incorporation of the subject matter of claim 27, from which claim 40 originally depended. As such, claim 40 is not vague and indefinite and, therefore, it is respectfully requested that this ground of rejection be removed.

In view of the amendments and the above remarks, it is submitted that the claims clearly set forth the metes and bounds of the subject matter regarded as the invention. Accordingly, it is respectfully requested that the rejection of claims 3 and 40 under 35 U.S.C. §112, second paragraph be removed.

F. Rejection Under 35 U.S.C. §102(b)

The rejection of claims 2 to 5, 16 to 19 and 40 under 35 U.S.C. §102(b) as allegedly anticipated by the Hanas, et al. reference is respectfully traversed.

The present invention provides variants of zinc finger proteins wherein the finger modules of the variant have been modified. Applicants teach isolation of modified zinc finger-proteins that bind to a cellular nucleotide sequence different from the cellular nucleotide sequence which it bound prior to modification of the variant. (See application, page 5, lines 23-30.)

Hanas et al. describe mutation of TFIIIA by internal deletion, which either eliminates the fourth zinc finger of the protein or results in a fusion of the seventh and eighth fingers of TFIIIA. Hanas et al. show that the resulting mutants are capable of promoting 5S gene transcription. (See

pages 9864-9865 of Hanas et al.) Hanas et al. do not teach or suggest that the mutated proteins might serve an additional function in gene expression.

The present invention does not require that the variant retain the transcription regulatory activity of the protein prior to mutation or modification. As such, Hanas et al. do not disclose all of the elements of the claimed invention. Accordingly, it is respectfully requested that the rejection of claims 2 to 5, 16 to 19 and 40 under 35 U.S.C. § 102(b) be removed.

G. Rejection Under 35 U.S.C. §103

The rejection of claims 2, 4, and 16 to 17 under 35 U.S.C. §103(a) as allegedly anticipated by the Crozatier, et al. reference is respectfully traversed.

Crozatier et al. states that "*[i]n vitro* experiments to determine the consequences of the *sry* δ^{SF1}, *sry* δ^{SF2}, and *sry* δ^{14} mutations on the DNA recognition and binding properties of the *sry* δ protein are in progress." (Crozatier, et al., p. 915, col. 1.) Crozatier et al. do not teach or suggest that the various mutations might be useful for enhancing or repressing gene expression. Even if the teachings of Crotzier motivated one of skill in the art to isolate the mutant peptides of that reference and analyze them for their DNA-binding properties, one would not have had a reasonable expectation of success of obtaining a mutant useful for regulation of gene expression.

The claimed invention does not require that the vriant zinc finger polypeptides retain the binding activity before and after modification. Like Hanas et al., Crozatier et al. show that mutated proteins do maintain some of the ability to bind to DNA as possessed before the invention.

Crozatier et al., in combination with the knowledge of one of skill in the art, does not teach or suggest all of the limitations of the present invention. As such, claims 2, 4, and 16 to 17 of the present invention are not obvious in light of the teachings of Crozatier et al. Accordingly, it is respectfully requested that the rejection of claims 2, 4 and 16 to 17 under 35 U.S.C. §103(a) be removed.

III. CONCLUSION

In view of the foregoing, Applicants respectfully submit that the claims are in condition for allowance. Should there be any questions concerning this Amendment, the Examiner is respectfully invited to contact the undersigned.

Respectfully submitted,

Date: 5/31/01

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

3. The variant of claim 2, which is [derived from] a zinc finger-nucleotide binding polypeptide selected from the group consisting of Zif268 and TFIIIA.

40. [A] An isolated zinc finger-nucleotide binding polypeptide variant produced by [the method of claim 27] a method for isolating a zinc finger-nucleotide binding polypeptide variant which binds to a cellular nucleotide sequence comprising:

a) identifying the amino acids in a zinc finger-nucleotide binding polypeptide that bind to a first cellular nucleotide sequence and modulate the function of the nucleotide sequence;

b) creating an expression library encoding the polypeptide variant containing randomized substitution of the amino acids identified in step a) above;

c) expressing the library in a suitable host cell; and

d) isolating a clone that produces a polypeptide variant that binds to a second cellular nucleotide sequence and modulates the function of the second nucleotide sequence,

wherein the variant is comprised of at least two zinc finger modules and wherein the amino acid sequence of at least one module that binds the second nucleotide sequence comprises two cysteines and two histidines whereby both cysteines are amino proximal to both histidines and wherein at least one of the at least two modules of said variant has at least one amino acid sequence modification.

Please add the following new claims:

--42. (New) A hybrid zinc finger protein that binds to a target nucleic acid, the hybrid zinc finger comprising zinc fingers from a first protein linked to zinc fingers from a second protein.

43. (New) The hybrid zinc finger protein of claim 42, wherein at least two of the zinc fingers are variant zinc fingers.

44. (New) The hybrid zinc finger protein of claim 42, wherein the variant zinc fingers are mutagenized forms of natural zinc fingers.

45. (New) The hybrid zinc finger protein of claim 42 that modulates expression of the target nucleic acid.

46. (New) The hybrid zinc finger protein of claim 42, wherein the amino acid sequence of each zinc finger comprises two cysteines and two histidines whereby both cysteines are amino proximal to both histidines.

47. (New) A method for isolating a zinc finger protein that binds to a target nucleic acid, comprising:

a) creating an expression library encoding variant zinc finger proteins, wherein the variant zinc finger proteins each comprises a plurality of zinc fingers, each zinc finger comprising two cysteines and two histidines whereby both cysteines are amino proximal to both histidines and an alpha helix is within the region bordered by the outermost cysteine and histidine residues, and the zinc finger proteins differ from each other in at least an amino acid immediately preceding the alpha helix and at alpha helix positions +1, +2 and +6 of at least one zinc finger;

b) expressing the library in a suitable host cell; and

c) isolating a zinc finger protein that binds to a desired target nucleic acid, or a nucleic acid encoding the zinc finger protein.

48. (New) The method of claim 47, wherein the expression library is a phage display library.

49. (New) The method of claim 47, wherein the variant zinc finger proteins differ from each other in at least an amino acid immediately preceding the alpha helix and at alpha helix positions +1, +2 and +6 of at least two zinc fingers.

50. (New) The method of claim 47, wherein the variant zinc finger proteins differ from each other by random substitutions.--

A check in the total amount of $1,130.00 is enclosed. This includes $890.00 for the fee to file this Petition for Three-Month Extension, and $240.00 for surcharge for filing Information Disclosure Statement after receipt of office action. The Commissioner is hereby authorized to charge any additional fees required by this filing, or credit any overpayment, to Deposit Account No. 50-1355. A duplicate copy of this Petition is enclosed.

Respectfully submitted,

Date: 5/31/01

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